Maximising the utility of bioelectrical impedance analysis for measuring fish condition requires identifying and controlling for sources of error

Curtis Champion[a,b,*], Alistair J. Hobday[b,c], Gretta T. Pecl[a,c], Sean R. Tracey[a,c]

[a] Institute for Marine and Antarctic Studies, Hobart, Tasmania 7001, Australia.
[b] CSIRO Oceans and Atmosphere, Hobart, Tasmania 7000, Australia.
[c] Centre for Marine Socioecology, Hobart, Tasmania 7001, Australia.

Abstract

Body condition indices are commonly used to represent the physiological status of fishes. Bioelectrical impedance analysis (BIA) has emerged as a rapid, nonlethal and cost-effective method for measuring fish condition and predicting proximate composition components, such as per cent fat. Measuring the condition of fish obtained from varied sources requires consideration of potential sources of error to ensure robust and comparable data are obtained. This is important when opportunistically applying BIA to assess fish condition for species that are logistically difficult to sample (e.g., large-bodied marine fishes), when different sampling methods are used, or where fish handling effects may confound condition comparisons. We experimentally tested the effects of five factors related to fish handling on an instantaneous body condition index (phase angle) measured using BIA. Using the coastal-pelagic yellowtail kingfish (*Seriola lalandi*) as a model species, we identified significant effects for four out of five factors tested: time since death, temperature of the tissue, removal of the gills and gastrointestinal tract, and the anatomic location for measurements. We propose protocol considerations when using BIA to opportunistically measure condition in fish obtained from varied sources. These sampling protocols for the robust application of BIA can maximise the utility of this approach for opportunistically measuring body condition in fish.

Keywords: bioelectrical impedance analysis, citizen science, fish condition, fisheries, physiological status, sampling design

*Corresponding author:
Curtis Champion
email: curtis.champion@utas.edu.au

1. Introduction

Body condition indices in fish and fisheries research are widely used to infer physiological status (Murphy et al., 1990). Measures of fish condition can reveal important biological and ecological relationships, such as variation in growth and recruitment of spatially discrete stocks (Rätz and Lloret, 2003) and the influence of abiotic factors on the physiology of fishes (Adams et al., 2018; Kjesbu et al., 2014). Given that environmental variables are known to influence fish condition (Willis and Hobday, 2008; Champion et al., 2020), and that climate-driven oceanographic changes are rapidly occurring globally (Wu et al., 2012), body condition indices are a useful approach to assess species' responses to environmental change (Miranda et al., 2019).

Researchers use either traditional (Murphy et al., 1990) or emerging methods (Hartman et al., 2015) for quantifying the physiological status of fishes. Traditional measures of fish condition, such as Fulton's K and relative weight (W_r), typically rely on deriving species-specific length–weight relationships and measuring individual deviations from expected values (Hampton, 1986). However, these measures have been criticised as inaccurate estimates of physiological status (Green, 2001), subsequently casting doubt over their ecological relevance. For example, the tendency of fish to replace lipids with water when fatigued or losing energy (Love, 1970) is likely to mask any true reduction in body condition when total mass based condition estimates are applied (Hartman and Margraf, 2008). Direct approaches for measuring body composition indices, such as per cent fat or energy content (e.g., bomb calorimetry), are effective but are not widely applicable due to expensive and labour intensive laboratory processing requirements and the need for fish to be euthanised (Vogt et al., 2002). Alternatively, novel electrical conductivity methods have emerged as promising techniques capable of measuring the body condition of fishes quickly and nonlethally (Hartman et al., 2015). These techniques include total body electrical conductivity and bioelectrical impedance analysis (BIA), which rely on measuring the impedance of biological tissue to an imperceivably weak electrical current. BIA originated in medical fields and is highly effective for measuring human body composition (e.g., fat content and total water) (Dittmar, 2003; Lukaski et al., 1985) and nutritional status (Barbosa-Silva et al., 2003). BIA is also an accurate predictor of body composition in animals (Marchello et al., 1999; Tierney et al., 2001), and is particularly useful in fish and fisheries research because the instrument is portable and user-friendly (Cox and Hartman, 2005), allowing measures of the electrical impedance of biological tissue under a range of field and laboratory conditions and for fish of varying morphologies (Hartman et al., 2015). Given that the majority of studies to date have applied BIA to anadromous fishes (e.g., Salmonids) or in aquaculture settings (Cox and Heintz, 2009; Cox and Hartman, 2005; Duncan et al., 2007), there is a need to investigate practical considerations for using BIA to measure the body condition of marine fishes in the field.

Multiple studies have demonstrated that the composition and condition of fish can be accurately quantified with BIA using direct measures (i.e., resistance and reactance) and measures derived using electrical equations (Cox and Heintz, 2009; Hartman et al., 2015). To date, the majority of studies applying BIA to fish have relied on developing correlative relationships between the electrical impedance of tissue and measures of proximate body composition, such as total fat, per cent ash and moisture content (Cox and Hartman, 2005; Duncan et al., 2007; Hafs and Hartman, 2011). While BIA is often proposed as a nonlethal method for determining body composition and condition, proximate analyses required for calibrating correlative models rely on euthanising a representative sample of individuals (Cox and Hartman, 2005). Once the relationships are calibrated, there is no need to sacrifice more animals and estimates of proximate body composition and condition can be made in approximately the same time it takes to measure fish length. Guidelines for model calibration have been established by Hartman et al. (2015), who suggest that a minimum of 60 individuals

are required for proximate composition analyses and biochemical assays to develop accurate predictive relationships. However, 60 samples may be a prohibitively high number for many species, for example in studies seeking to apply BIA to threatened species or those that are logistically difficult to sample, such as large pelagic fishes. Furthermore, the calibration of predictive models requires sufficient variation in response metrics (e.g., per cent fat) that may not be attainable when opportunistically sampling wild fishes. It is also uncertain how applicable relationships between impedance measurements and proximate composition measures are to individuals sampled from different ecological and spatiotemporal contexts than those used to calibrate these relationships. There is a need to investigate the application of BIA for instantaneously quantifying fish condition in the field and without model calibration given the potential for BIA to be utilised for opportunistically measuring fish obtained from a range of sources (e.g., citizen science initiatives, recreational fishers, commercial landings).

Deriving robust body condition data through the application of BIA in the field requires an understanding of potential sources of measurement error. Measurement error can arise from (1) incorrectly using the BIA instrument to take measurements or, (2) a combination of factors relating to how and when BIA measurements are taken, which may be unique to individual species or groups of closely related species. Past studies have indicated practices that are important for correctly utilising BIA tools (Hartman et al., 2015). These include blot drying fish prior to making contact with the BIA instrument's electrodes, the application of firm and steady pressure between electrodes and fish tissue to establish a strong electrical circuit, and placing fish on a nonconductive board to ensure that the electrical circuit is not affected by external conductive material (Cox and Hartman, 2005; Hartman et al., 2015). However, additional sources of measurement error may arise from species-specific factors, such as the anatomic location that electrodes are placed on individuals when measuring biological impedance. For example, Cox and Heintz (2009) observed significant differences between phase angle values (a body condition index derived from BIA measurements detailed in the methods section) taken along the dorsal and ventral sides of chum salmon (*Oncorhynchus keta*), suggesting that the anatomic location of BIA measurements should be consistent in studies undertaking comparative analyses. Temperature has also been shown to influence BIA measurements (Cox et al., 2011; Gudivaka et al., 1996). Specifically, phase angle values recorded for dead fish have been shown to increase as tissue temperature decreases (Cox and Heintz, 2009). These findings suggest that controlling for the effects of ambient temperature on BIA measurements is likely to be an important consideration for field-based studies that aim to compare data from locations or times with varying environmental temperatures.

The nature of the sampling program being undertaken can also introduce error. Sampling dead individuals is common and, given that cells begin to break down post-mortem, another possible source of error is the time after death that measurements are taken (Cox et al., 2011). For example, BIA measurements taken at varying times since fish have been caught and killed may not be comparable due to the degradation of biological tissue post-mortem. Analysing Coho Salmon (*Oncorhynchus kisutch*), Cox et al. (2011) found that BIA measurements became significantly different from freshly sampled individuals after fish had been dead for nine hours (while being held on ice). This is likely to be an important source of error when applying BIA in fishery-dependent sampling programs, including citizen science initiatives that encourage the donation of samples from recreational or commercial fishers. This window of time is likely to be species-specific due to the influence of variation in fish physiology on BIA measurements. Subsequently, the development of robust field-sampling protocols requires information from experiments that quantitatively evaluate potential sources of error to ensure that BIA measurements taken on fish from varying sources are representative of the condition of live individuals and measurements are comparable.

Despite evidence that several sources of measurement error can confound comparative analyses of BIA data, assessing different sources of error for the purpose of informing the robust application of BIA remains ad hoc. Furthermore, past studies (e.g., those reviewed by Hartman et al. (2015)) have a strong focus on small-sized anadromous fishes in laboratory settings and the responses of medium-bodied marine fishes (~50-100 cm) in a field setting may vary. Experiments that test for sources of measurement error are an important step prior to field studies that seek apply BIA, particularly to samples donated by citizen scientists (e.g., at the conclusion of fishing competitions or out of interest in, and desire to contribute to, scientific projects). This step is necessary to ensure that studies yield robust and comparable body condition data that can be used to address ecological hypotheses.

1.1 Objectives

The objective of this study was to identify factors that introduce measurement error in field-based studies that seek to opportunistically apply BIA to samples not captured by a research team. This step should occur prior to undertaking a field study where fish of different processing history or style may be encountered. Utilising the medium-sized coastal-pelagic yellowtail kingfish (*Seriola lalandi*; hereafter 'kingfish'), we tested the potential effects of: (1) time since death, (2) fish size, (3) gill and gastrointestinal tract removal, (4) anatomic location of measurement, and (5) temperature of tissue on an instantaneous body condition index (phase angle) that is derived from BIA measurements. The results of these experiments informed a protocol for obtaining comparable phase angle data when applying BIA to samples from varying origins. We seek to assist researchers and managers to develop robust sampling protocols for the field-based application of BIA to their species of interest.

2. Methods

2.1 Bioelectrical impedance analysis

Bioelectrical impedance analysis works by passing a high frequency current (50 kHz) of imperceptible amplitude (800 μA) through body tissue between signal and receiver electrodes that are either pressed against the skin or inserted less than 1 cm into body tissue (depending on the configuration of the BIA tool) to measure impedance (Cox and Hartman, 2005). Impedance is the sum of two vectors of electrical current, resistance and reactance, which are measured directly by the BIA tool. Resistance and reactance values are indicative of physiology status and can be used to derive additional biologically relevant parameters using electrical equations (Hartman et al., 2015). Resistance measures the ability of extracellular material to conduct electricity (Cox and Hartman, 2005). This is achieved in BIA by using an electrical current that is incapable of passing through cellular membrane, due to the presence of the nonconductive lipid bilayer that is pressed between two conductive protein layers. Subsequently, resistance reflects extracellular material, such as fat, which is nonconductive and can be indicated by higher resistance values (Cox et al., 2011). Reactance is the ability of a substance to hold a charge and is used in BIA to measure opposition of the cellular lipid bilayer to an alternating current (Cox et al., 2011). Subsequently, reactance is a measure of the total volume of healthy cells, which is indicative of an individual's body condition (Kyle et al., 2004).

2.1.1 Electrical phase angle as a body condition index

Electrical phase angle is a metabolic condition index (Willis and Hobday, 2008) that is determined by the angle between the two vector components of impedance (resistance and reactance) and is defined as:

$$\text{phase angle } (^\circ) = \left(\arctan\left(\frac{X_c}{R}\right)\right) \times \frac{180^\circ}{\pi}$$

where X_c is reactance (ohms) and R is resistance (ohms). Phase angle measurements ranges from 0° to 90°, where higher values indicate good body condition due to high readings of X_c that are indicative of large quantities of intact cell membranes (Foster and Lukaski, 1996).

Unlike other body composition indices linked to BIA measurements (e.g., per cent fat), phase angle values can be instantaneously derived from resistance and reactance measurements and avoids the need to euthanise a representative sample of individuals to calibrate regression equations (Cox and Hartman, 2005). The use of phase angle instead of regression analysis for describing composition variables has become common in medical fields because phase angle is linked to metabolic rate and nutritional status, and can thus be used as a direct measure of body condition (Barbosa-Silva et al., 2003). In pelagic fish, Willis and Hobday (2008) used phase angle data to describe the body condition of southern bluefin tuna (*Thunnus maccoyii*) across years. Furthermore, Cox and Heintz (2009) found that phase angle was effective in differentiating between states of body condition in a variety of salmonids, where angles < 15° were judged to indicate fish in poor condition and angles > 15° indicated fish that were in relatively good condition. Therefore, phase angle is a promising metric because it provides an informative measure of fish condition that is instantaneous, nonlethal and does not require model calibration, thus eliminating uncertainty surrounding parameter estimates from regression analyses.

2.2 Sampling of study species

To assess for potential source of error that may influence phase angle values measured during opportunistic field-based sampling, kingfish were sampled from south-eastern Australia using hook-and-line fishing between November 2016 and February 2019 for experimental analyses. Kingfish were chosen so that the experimental results herein could be used to inform a broader ecological study that aimed to measure the body condition of this species across a gradient of oceanographic habitat suitability (Champion et al., 2020). Kingfish from south-eastern Australia represent a single, genetically distinct population (Miller et al., 2011) with a distribution that is influenced by oceanographic variables, including sea surface temperature, sea level anomaly and current velocity (Brodie et al., 2015; Champion et al., 2018). This species is targeted in several eastern Australian fisheries, where the estimated annual recreational catch exceeds the average annual commercial catch (Henry and Lyle, 2003; Lowry et al., 2016). Therefore, kingfish are representative of species that may be donated by recreational fishers to scientific research projects seeking to quantify fish condition.

2.3 Experiments

2.3.1 Experiment 1 – time since death

Variation in the time between capture (fish death) and when BIA measurements are taken may compromise accurate body condition comparisons (Cox et al., 2011). To test the effect of time since the death of fish on phase angle measurements, kingfish (n = 46) were caught by hook-and-line fishing, killed via ikejime (i.e., pithing), and held on ice and subjected to repeated phase angle measurements that were taken at 5-hour intervals for a period of 120 hours. Preliminary data suggested that significant differences in phase angle measurements were apparent within the first 48 hours of fish being killed and held on ice, so measurements were taken at 10-hour intervals after fish had been repeatedly measured at 5-hour intervals for the first 70 hours of the experiment. Kingfish were caught over three consecutive austral summer seasons between December 2016 and February 2019 and this experiment was repeated on three separate occasions (i.e., each summer) to maximise sample size and due to logistical constraints associated with holding more than ~15 individuals on ice simultaneously. All fish were covered with ice and kept in a 200-litre ice box, as is common practice when kingfish are caught in commercial and recreational fisheries. Phase angle measurements were taken along the dorsal

musculature of kingfish (location A, Fig. 1) placed in a left-facing orientation on a nonconductive polyethylene board. Fish were removed from the ice box for a period of 10 – 30 seconds for each phase angle measurement. A TP20 digital thermometer (ThermPro, Toronto, Canada) was placed inside the ice box and readings were recorded 5-hourly in conjunction with BIA measurements to ensure that temperature remained constant throughout the duration of the experiment. Fresh ice was applied when necessary in order to maintain a consistent temperature (5°C ± 0.5°C) irrespective of the outside ambient temperature.

A linear mixed effects modelling approach was applied to assess for an effect of time after death on phase angle measurements, which has the form (in script notation):

Phase angle = time since fish death + (1|*fish ID*)

where phase angle is a body condition index (0 – 90°) modelled as a function of time since fish death (hours; continuous variable), with individual fish identity (fish ID) fitted as a random intercept term. Paired sample pairwise comparisons were applied using the 'multcomp' package (Hothorn et al., 2008) in the R programming language (R Core Team, 2017) to identify the time at which fish death began to significantly affect phase angle measurements. Pairwise comparisons applied Bonferroni corrections to reduce the probability of Type 1 error due to multiple testing (Cabin et al., 2000). This post hoc analysis treated 'time after death' as a categorical variable and tested for significant differences between phase angle measurements taken at time = 0 (i.e., the time of death) and all subsequent time points when phase angle measurements were taken during the experiment. Phase angle measurements taken after kingfish had been held on ice for 5 hours were also compared with measurements taken at all subsequent time points. This was done to account for the potential effects of the temperature of fish tissue on phase angle measurements (Hartman et al., 2011), which varied between measurement taken at time = 0 hours (i.e. freshly caught fish) and time = 5 hours (i.e. 5 hours spent on ice).

2.3.2 Experiment 2 – fish size

Fish size may confound body condition comparisons using BIA due to potential ontogenetic changes in the body composition of fishes (Pilati and Vanni, 2007). To test for a relationship between fish size and phase angle, measurements were taken on kingfish (n = 98) that ranged from 29 – 127 cm FL and 0.41 – 20.1 kg along the dorsal musculature (location A, Fig. 1) of individuals place in a left-facing orientation on a nonconductive polyethylene board. Because variation in environmental temperature has been shown to influence BIA measurements (Hartman et al., 2011), all fish were held on ice for 60 minutes after death and prior to measurement to control for the potentially confounding effects of temperature on phase angle measurements. Relationships between electrical phase angle measurements and the length and weight of kingfish were analysed using simple linear models to test if slopes significantly differed from zero. Nine individuals were removed from the full dataset as these contributed to a violation of the assumption of homoscedasticity of variance, resulting in the final linear model being fitted to n = 87 independent measurements.

2.3.3 Experiment 3 – gilled and gutted fish

Removing the gills and gastrointestinal tract of fish is common practice in recreational and commercial fisheries to preserve seafood quality and this procedure is likely to be encountered when applying BIA to fish opportunistically provided by fishers. To test for an effect of removing the gills and gastrointestinal tract on phase angle values, BIA measurements were taken along the dorsal musculature and ventral tissue (locations A and B, respectively, Fig. 1.) of kingfish (n = 11) before and after the removal of these tissues. Recently caught kingfish were held on ice for 60 minutes prior to measurement to control for the potentially influence

of temperature variation on phase angle values. All measurements were taken on a nonconductive polyethylene board with fish in a left-facing orientation. Paired sample *t*-tests were applied to assess if phase angle measurements were significantly affected by the removal of the gills and gastrointestinal tract. Separate analyses were applied to phase angle data taken along the dorsal musculature and ventral tissue of kingfish to control for potential differences due to the anatomic location of measurements (Cox et al., 2011). Effect sizes pertaining to gilling and gutting fish were quantified using Cohen's *d*, where values of ~0.2, ~0.5 and >0.8 represent small, medium and large effect sizes, respectively (Cohen, 1988). Given that the sample size available for this experiment was low relative to other experiments presented herein, we also undertook standard power and sample size analyses to quantify: 1. the likelihood of detecting significant differences given the available sample size, and 2. sample sizes required to achieve high levels (i.e. 0.8 and 0.9) of statistical power (Hastie et al., 2001).

2.3.4 Experiment 4 – anatomic location of measurement

The anatomic location of signal and receiver electrodes has been shown to influence BIA readings in pink salmon (*Oncorhynchus gorbuscha*; n = 5, mean fork length = 49.4 cm, SD = 0.9cm) (Cox et al., 2011). To test if this effect is consistent in the medium-bodied, coastal-pelagic kingfish, we compared phase angle measurements taken along the dorsal musculature and ventral tissue of individual kingfish ranging from 42 – 127 cm FL and 1.1 – 20.1 kg (n = 25; Fig. 1.). All fish were measured in a left-facing orientation on a nonconductive polyethylene board after being held of ice for 60 minutes following death. The BIA unit that was used had a fixed distanced (10 cm) between signal and receiver electrodes and this desistance was therefore consistent for measurements taken at different locations. A paired sample *t*-test was applied to assess if phase angle measurements were significantly affected by the anatomic location of electrodes. As in Experiment 3, Cohen's *d* was quantified to estimate the effect size between phase angle measurements recorded at different anatomic locations.

2.3.5 Experiment 5 – temperature effects

Previous studies have found that approximately 10°C of temperature change can significantly effect BIA measurements (Hafs and Hartman, 2015; Hartman et al., 2011). To assess if this trend was consistent for phase angle measurements taken on kingfish, five individuals were killed and placed on ice with the probe of a TP20 digital thermometer (ThermPro, Toronto, Canada) inserted 2 cm into the dorsal musculature of each individual. Temperature and phase angle measurements were taken at 10-minute intervals over a period of 2 hours. The temperature of fish tissue declined throughout the experiment as a result of fish being taken from an ambient environmental temperature (~20°C) and placed on ice. To test for an effect of temperature on phase angle measurements, a linear mixed effects model was fitted to repeated measures data recorded for each individual throughout the duration of the experiment, which has the form (in script notation):

$$\textit{Phase angle} = \textit{temperature} + (1|\textit{fish ID})$$

where phase angle is a body condition index (0 – 90°) modelled as a function of temperature (°C), with individual fish identity (fish ID) fitted as a random intercept term.

All BIA measurements were taken using the Seafood Analytics Certified Quality Reader (CQ Foods, Inc., Clinton Township, MI, USA) by study author C.C., as user experience can also affect BIA readings (Cox et al., 2011). Data from all experiments were analysed using the R programming language (R Core Team 2017). For all analyses, diagnostic plots were used to assess if the assumptions of normality and homogeneity of variance were satisfied. Kingfish were sampled in accordance with the University of Tasmania's Animal Care and Ethics approval number A0016150.

3. Results

3.1 Experiment 1 – time since death

Phase angle measurements taken along the dorsal musculature of kingfish that were held on ice declined at a rate of 0.085 degrees hour^{-1} (0.081-0.088 95% CI; $t_{1,916}$ = -48.4, $P < 0.001$; parameters for fixed component of the linear mixed effects model: int = 31.239, slope = −0.085; intraclass correlation coefficient: Fish ID = 0.779). Paired sample pairwise post hoc tests revealed that phase angle measurements were significantly different between kingfish that had been held on ice for 20 hours and kingfish measured at the time of death (i.e., time since death = 0 hours; $P = 0.029$), and the statistical significance of this difference became increasingly greater as time since death increased beyond 20 hours (Fig. 2). Comparisons between repeated phase angle measurements taken on kingfish that had been held on ice for 5 hours and measurements taken at all subsequent time points also identified that significant differences occurred after 20 hours ($P = 0.020$).

3.2 Experiment 2 – fish size

Linear regression analysis showed no significant relationship between phase angle measurements and the length or weight of kingfish (length: $F_{1, 87} = 0.849$, $P = 0.36$, $r^2 < 0.01$, Fig. 3a; weight: $F_{1, 87} = 2.673$, $P = 0.21$, $r^2 < 0.01$, Fig. 3b).

3.3 Experiment 3 – gilled and gutted fish

Phase angle measurements were significantly reduced due to the removal of the gills and gastrointestinal tract of kingfish (Fig. 4). Specifically, the removal of the gills and gastrointestinal tract resulted in significant declines in phase angle measurements taken along both the dorsal musculature (paired sample *t*-test: $t_{10} = 9.99$, $P < 0.001$) and ventral tissue (paired sample *t*-test: $t_{10} = 11.99$, $P < 0.001$) of kingfish. Larger reductions in phase angle data were recorded for measurements taken along the ventral tissue of kingfish (Δ mean phase angle = -7.3; Cohen's $d = 1.38$) when compared with measurements taken along the dorsal musculature (Δ mean phase angle = -3.2; Cohen's $d = 0.69$). The statistical power associated with dorsal and ventral measurements was 0.65 and 0.94, respective ($n = 11$). Sample size analyses revealed that dorsal measurements require sample sizes of $n = 17$ and $n = 24$ to achieved statistical power of 0.8 and 0.9, respectively, provided that mean differences between before and after measurements and the standard deviation these pooled data remained consistent. Sample size analyses for ventral measurements demonstrated that sample sizes of 7 and 9 are likely to be sufficient to detect true significant difference in 80% and 90% of instances, respectively.

3.4 Experiment 4 – anatomic location of measurement

Phase angle values depended on the anatomic location of measurement (Fig. 5). Specifically, phase angle was significantly higher when measured across the dorsal musculature of kingfish when compared to measurements taken across the ventral tissue of individuals (paired sample *t*-test: $t_{24} = 9.91$, $P < 0.001$; Cohen's $d = 0.95$).

3.5 Experiment 5 – temperature effects

The temperature of kingfish tissue was found to have a significant negative effect on phase angle measurements ($t_{1,56}$ = -11.58, $P = 0.008$; fixed components of the linear mixed effects model: int = 28.10, slope = −0.11; intraclass correlation coefficient: Fish ID = 0.986). Phase angle values were found to stabilise at temperatures less than approximately 5°C, or after being held on ice for approximately 60 minutes (Fig. 6)

4. Discussion

Testing for potential sources of measurement error is a crucial step in assessing the utility of novel research tools and for developing sampling protocols that yield comparable data. As interest in the application of BIA to fish continues to increase (Hartman et al., 2015), practical approaches to control for sources of variation are essential to ensure that BIA can be widely applied as a low cost, instantaneous and nonlethal approach for measuring fish condition. For example, blot drying and measuring fish on a nonconductive board is a standard practices for avoiding measurement error that should be adopted in all applications of BIA to fish and fisheries research (Cox and Hartman, 2005). Given that BIA is well-suited for instantaneously and nonlethally assessing fish condition (Willis and Hobday, 2008), our experiments focused on factors that may influence the application of BIA in the field and to species that are difficult to obtain for traditional condition or proximate composition analyses (e.g., medium-sized pelagic fishes). While these experiments demonstrate factors that can confound comparisons of phase angle data that are opportunistically collected from different sources, they also highlight practical measures to effectively control for sources of variation. Here we place our results in the context of protocol considerations for opportunistically deriving comparable phase angle measurements from sources where fish handling differences may influence data quality (Table 1).

4.1 How long after death can comparable phase angle measurements be taken?

Our results indicate that phase angle measurements taken along the dorsal musculature of kingfish did not significantly change in fish that were placed on ice for 15 hours or less. These findings are comparable with those of Cox and Heintz (2009), who did not find an effect of time on phase angle measurements taken on juvenile coho salmon within 12 hours of death. Similarly, Cox et al. (2011) also investigated the effect of time since death on coho salmon and found that both vector components of impendence, resistance and reactance, can be reliably measured within 9 hours of death provided fish are held on ice. The reduction in phase angle values through time can be attributed to the effects of rigor mortis (muscle contraction) on the integrity of cell membranes, which results in their degradation and the subsequent release of electrolytes and water into extracellular space (Martinsen et al., 2000). This process affects the ratio of intact cell membrane to extracellular material within fish tissue, which is used to calculate phase angle, and ultimately results in a negative relationship between time since death and phase angle values. Because icing fish delays post-mortem rigor mortis and subsequent tissue breakdown (Orr, 1920), emphasis should be placed on the importance of icing fish immediately following death to maximise opportunities to accurately measure fish condition using BIA (Cox and Heintz, 2009). Importantly, our results highlight that when fish are placed on ice following capture, an adequate amount of time is likely to be available to researchers to enact the logistics required to opportunistically sampling fish caught by recreational or commercial fishers (e.g., up to 15 hours for kingfish).

4.2 Does the removal of the gills and gastrointestinal tract affect phase angle?

Removing the gills and gastrointestinal tract soon after capturing fish is commonly undertaken to preserve the seafood quality of species targeted in recreational and commercial fisheries (Haard, 1993). Our results found that this practice significantly effects phase angle measurements taken along both the dorsal musculature and ventral tissue of kingfish. Greater statistical power was associated with comparisons measured along the ventral tissue of kingfish (0.94) relative to comparisons made using measurements taken along dorsal musculature (0.65). However, taken together these findings indicate that phase angle measurements are only comparable within groups of individuals that have had gills and gastrointestinal tract removed, or within groups of intact individuals. Whenever possible, we recommend taking phase angle

measurements prior to the removal of the gills and gastrointestinal tract due to variability in the amount of tissue removed when fish are processed due to, for example, different techniques used by fish processors. Greater differences were found between before and after phase angle measurements taken along the ventral tissue of kingfish, which is the anatomic location associated with the greatest tissue loss when removing the gills and gastrointestinal tract, than for measurements taken along the dorsal musculature. This indicates that impedance measurements along the ventral tissue are most sensitive to the effects of gill and gastrointestinal removal, suggesting that phase angle should be measured along the dorsal musculature of fish that have undergone processing to best control for these effects.

4.3 How does the anatomic location of measurement and fish size affect phase angle?

Consistent with previous studies showing that BIA measurements are specific to the anatomic location of electrode placement (Cox et al., 2011; Hafs and Hartman, 2011), phase angle was significantly greater for measurements taken along the dorsal musculature than for measurements taken along the ventral tissue of kingfish. These differences are due to variation in the type of tissue present at dorsal and ventral locations and the ability of resistance and reactance measurements to differentiate between tissue types. The sensitivity of impedance measurements to varying tissue types (e.g., skeletal muscle, nervous tissue, kidney tissue, fat and bone) has been known of decades (Geddes and Baker, 1967), and our results strengthen the body of evidence that demonstrates the need to control for anatomic location when deriving comparable biological body condition data (Cox and Heintz, 2009; Cox et al., 2011; Hafs and Hartman, 2011). Given that organs within the peritoneal cavity of fish undergo ontogenetic changes (e.g., due to growth and spawning) (Van Aerle et al., 2004), it is pragmatic to take impedance measurements along the dorsal musculature of fish to minimise these effects on phase angle comparisons. Phase angle measurements taken along the dorsal musculature of kingfish were not affected by fish length or weight, suggesting that this location is most suitable for taking comparable phase angle measurements on fish of varying sizes.

4.4 Can icing fish post-capture control for temperature effects on phase angle measurements?

The effect of temperature on impedance measurements (Buono et al., 2004; Cox et al., 2011; Hartman et al., 2011; Stolarski et al., 2014) may be the primary limitation to opportunistically applying BIA in the field, particularly for comparing the body condition of species that occupy broad thermal niches. For example, temperature was found to have a significant negative effect on resistance and reactance measurements taken on tailor (*Pomatomus saltatrix*) that were held at 15°C and 27°C (Hartman et al., 2011). Similarly, phase angle measurements in pink salmon were slightly effected over an 8°C temperature range (Cox and Heintz, 2009). The influence of temperature on impedance measurements has prompted research into the development of correction equations to account for variation in temperature when using BIA to predict proximate body condition indices, such as per cent dry mass (Hafs and Hartman, 2015). However, it remains unclear if impendence measurements taken within relatively small temperature ranges (i.e., 1-2°C) are comparable (Cox and Hartman, 2005) and if practical solutions, such as icing fish for short periods of time after capture as suggested by Cox and Heintz (2009), can control for temperature effects. We found that phase angle measurements taken along the dorsal musculature of kingfish declined with temperature when repeated measurements were taken over approximately 20°C of temperature variation. Importantly, phase angle values were found to stabilise once the tissue temperature of kingfish declined to approximately 5°C or after approximately 60 minutes of fish being held on ice. These findings support the notion that the effects of temperature on phase angle can be controlled by icing fish for a short period of time (e.g. 1 hour) post-capture (Cox and Heintz, 2009), and highlight this

as a practical solution to control for temperature effects during field-based sampling (such as different water temperatures where fish were captured).

While our experiments highlight crucial considerations when developing protocols for the application of BIA to fish, the results are specific to kingfish and may not be transferable to other species due to the effects of variation in morphology and anatomic location of specific tissue types (Barlow, 1961). In general, icing whole fish following capture and death, and taking dorsal measurements after 1 hour should lead to accurate and comparable data (Table 1). If this is not possible, researchers should examine potential biases using experiments that evaluate sources of variation for species of interest. Following the sampling protocol summarised in Table 1 will produce robust phase angle measurements that are (1) directly relevant to studies investigating *Seriola* spp., (2) relevant to studies applying BIA other medium- to large-bodied coastal-pelagic fishes, and (3) comparable with future studies that investigate other fishes.

4.5 Additional considerations

Additional factors that were not experimentally investigated within this study may also influence phase angle measurements when opportunistically applying BIA in the field. Of particularly relevance to species caught in recreational and commercial fisheries is physiological stress associated with capture (Hartman et al., 2015), which varies depending on how fish are caught and killed. For example, the recreational capture of large pelagic fishes is commonly associated with long angling durations (> 10 minutes) that can leave fish in poor condition once landed (Tracey et al., 2016). However, the advent of novel fishing technologies (e.g., automatic reels and line made from strong synthetic materials) means that fish can now be landed in shorter amounts of time and with less associated physiological stress. Thus, variation in physiological stress associated with angling duration may confound body condition comparisons using BIA. Similarly, fish that experience physiological stress associated with capture in certain gear types (e.g., gillnets) before being killed by a fisher may not be comparable with individuals caught using other methods (e.g., hook-and-line) and immediately killed. While physiological stress associated with the method of fish capture may influence impedance measurements, no attempts have been made to quantify this potential effect. In the interim it is pragmatic to standardise the method of fish capture, where possible, to minimise variation in physiological stress and maximise the comparability of impedance data.

Variation in reproductive status has the potential to influence impedance measurements due to large fluctuations in gonad size and associated changes in relationship between lipid and moisture content during spawning periods (Domínguez-Petit et al., 2010; Jonsson et al., 1997). Despite this expectation, Stolarski et al. (2014) did not find an effect of reproductive status on impedance measurements taken on Dolly Varden (*Salvelinus malma*) despite gonads being on average 30 times larger (by weight) in spawning than in nonspawning individuals. These results suggest that detecting an effect of reproductive status on impedance measurements depends on whether electrical pathways intersect gonadal tissue, and highlight that this is not always the case even when measurements are taken along ventral tissue (Stolarski et al., 2014). Therefore, it may be possible to control for potential effects of reproductive status on impedance measurements by prioritising anatomic locations (e.g., dorsal musculature) that are likely to avoid the interaction of electrical currents with fish testes and ovaries. In the absence of species-specific experiments, comparative body condition analyses using impedance data should aim to measure and control for reproductive status (e.g. categorising reproductive status and incorporating this variable into a mixed effects modelling framework). Regardless, BIA can handle variation in the spawning status of fish better than traditional morphometric-based condition indices as impedance measurements relate to the composition of fish tissue and are not influenced by the relationship between length and weight (Hartman et al., 2015).

Correction factors have been proposed to account for error arising from variation in factors that are known to affect BIA measurements (Cox et al., 2011; Stolarski et al., 2014; Hafs and Hartman, 2015). Temperature corrections have proven useful for reducing variability surrounding relationships between BIA measurements and laboratory-derived proximate composition indices (Stolarski et al., 2014; Hafs and Hartman, 2015). For example, Hafs and Hartman (2015) found that the application of temperature corrections to BIA models attempting to predict per cent dry mass reduced root-mean-squared error by an average of 32%. While correction factors are needed so that calibrated relationships between BIA measurements and proximate composition indices are useful in a variety of environmental contexts, developing these requires holding an adequate sample size of live individuals under experimental conditions. This is unlikely in situations where researchers do not have access to experimental facilities or when research projects are dependent on measurements taken on dead fish (e.g., Stolarski et al., 2014). In such cases, it is pragmatic to focus on measures that reflect relative physiological status (e.g., phase angle) rather than proximate composition, and to initially control for potentially confounding effects when taking measurements, rather than attempt to retrospectively correct for sources of error.

Understanding and controlling for factors associated with the handling of fish is crucial for the wide and robust application of BIA in fish and fisheries research. While studies have previously highlighted sources of error (Cox et al., 2011; Hafs and Hartman, 2011), our results demonstrate the influence of factors that are specific to the opportunistic application of BIA to fish obtained from varied sources. It is in this context that BIA is particularly valuable due to the suitability of this approach for measuring the condition of species that are logistically difficult to sample using mass-based condition measures, and for quickly measuring the condition of a large number of individuals (e.g., commercial fisheries landings). By showing that factors likely to be encountered when applying BIA to fish from varied sources can confound impedance datasets, we encourage prospective BIA users to control for sources of variation so that comparable body condition data are available for ecological and fisheries management applications.

Figure captions and Tables

Figure 1. Anatomical locations for placing electrodes when taking BIA measurements on kingfish, where A denotes the placement of signal and receiver electrodes along the dorsal musculature, and B denotes the placement of signal and receiver electrodes along the ventral tissue of fish. Image credit: Peter Gouldthorpe (Tasmanian Department of Industries, Parks, Water and Environment).

Figure 2. Boxplots summarising changes in phase angle values measured along the dorsal musculature of kingfish (n = 46) that were repeatedly measured over a period of 120 hours while being held on ice. Red asterisks denote mean values.

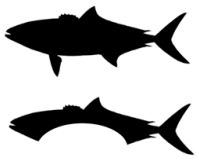
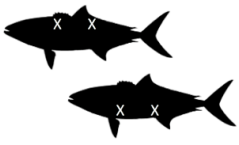
Figure 3. Relationships between fish size (a: fork length, and b: weight) and phase angle measurements taken along the dorsal musculature of kingfish (n = 98) that were held on ice for 60 minutes post-mortem. Red data points were removed from the final analysis as these markedly increased heteroscedastic of variance within this dataset. *NS* denotes non-significance at alpha = 0.05 level.

Figure 4. Boxplots summarising the distribution of phase angle measurements taken along (a) the dorsal musculature and (b) ventral tissue of kingfish (n = 11) before and after removal of the gills and gastrointestinal tract. Rugs on y-axes indicate phase angle values and red asterisks denote mean values.

Figure 5. Boxplots summarising the distribution of electrical phase angle values measured across the dorsal musculature and ventral tissue of kingfish (n = 25). Rugs on y-axes indicate phase angle values and red asterisks denote mean values.

Figure 6. Effect of temperature change on phase angle data repeatedly measured along the dorsal musculature of 5 yellowtail kingfish at 10-minute intervals for a period of two hours. Unique symbols represent individual fish and the black dashed line denotes the fixed slope of the linear mixed effects model.

572 Table 1. Key considerations, viable solutions and examples for deriving robust and comparable
573 phase angle data (referred to as 'condition measurements' within table) from varied sources
574 based on experiments undertaken herein and published literature.
575

	Consideration	Viable solution	Supporting evidence	Example in practice
Step 1	How long after fish death can accurate condition measurements be taken? 	Time since death affects condition measurements. Measurements taken within approximately 10 hours of fish death, provided fish are held on ice, should yield robust data.	Cox and Heintz, 2009 Cox et al., 2011 *Experiment 1* herein	Comparable condition measures were taken on kingfish (*Seriola lalandi*) between 0 and 15 hours since death while fish were held on ice (Champion et al., 2020).
Step 2	Will temperature affect condition measurements? 	Temperature affects condition measurements. Following capture and death, icing fish for short periods of time (e.g., 1 hour) prior to measurement can control for this effect.	Cox and Heintz, 2009 Cox et al., 2011 Hartman et al., 2011 Stolarski et al., 2014 *Experiment 5* herein	To yield comparable data, temperature effects have been controlled for by icing fish for 1 hour before taking condition measurements (Champion et al., 2020).
Step 3	Are condition measurements taken on whole fish comparable with fish that have been gilled and gutted? 	Condition measurements taken on whole fish are unlikely to be comparable with fish that have been gilled and gutted. Researchers should aim to compare measurements taken on whole fish only.	*Experiment 3* herein	In a comparison of the body condition of kingfish (*Seriola lalandi*) from eastern Australia, only whole fish were selected for sampling (Champion et al., 2020).
Step 4	Does the anatomic location of measurement affect condition data? 	Condition measurements taken at varying anatomic locations are unlikely to be comparable and a standardised location should be used.	Cox and Heintz, 2009 Cox et al., 2011 Hafs and Hartman, 2011 *Experiment 4* herein	Differences in impedance measurements taken at varying anatomic locations are known (e.g., Hafs and Hartman, 2011), and studies applying BIA commonly standardise the anatomic location of measurement (e.g., Stolarski et al., 2014).

576

Acknowledgements

We thank Derrick Cruz and Bobby May for assisting in the capture of kingfish. C.C. was supported by the Australian Society for Fish Biology's Michael Hall Student Innovation Award, The Holsworth Wildlife Research Endowment – Equity Trustees Charitable Foundation, and a postgraduate scholarship from the CSIRO – University of Tasmania Joint Quantitative Marine Science Program. G.P. was supported by an ARC Future Fellowship (FT140100596).

References

Adams, G.D., Leaf, R.T., Wu, W., Hernandez, F.J., Ojaveer, H.e.H., 2018. Environmentally driven fluctuations in condition factor of adult Gulf menhaden (*Brevoortia patronus*) in the northern Gulf of Mexico. ICES J. Mar. Sci. 75, 1269-1279.

Barbosa-Silva, M.C.G., Barros, A.J., Post, C.L., Waitzberg, D.L., Heymsfield, S.B., 2003. Can bioelectrical impedance analysis identify malnutrition in preoperative nutrition assessment? Nutr. J. 19, 422-426.

Barlow, G.W., 1961. Causes and significance of morphological variation in fishes. Syst. Zool. 10, 105-117.

Brodie, S., Hobday, A.J., Smith, J.A., Everett, J.D., Taylor, M.D., Gray, C.A., Suthers, I.M., 2015. Modelling the oceanic habitats of two pelagic species using recreational fisheries data. Fish. Oceanogr. 24, 463-477.

Buono, M.J., Burke, S., Endemann, S., Graham, H., Gressard, C., Griswold, L., Michalewicz, B., 2004. The effect of ambient air temperature on whole-body bioelectrical impedance. Physiol. Meas. 25, 119-129.

Cabin, R.J., Mitchell, R.J., 2000. To bonferroni or not to bonferroni: When and how are the questions. Bull. Ecol. Soc. Am. 81, 246-248.

Cohen, J., 1988. Statistical power analysis for the behavioral sciences, Second Edition. Routledge, Hillsdale, NJ, USA.

Champion, C., Hobday, A.J., Tracey, S.R., Pecl, G.T., 2018. Rapid shifts in distribution and high-latitude persistence of oceanographic habitat revealed using citizen science data from a climate change hotspot. Global Change Biol. 24, 5440–5453.

Champion, C., Hobday, A.J., Pecl, G.T., Tracey, S., 2020. Oceanographic habitat suitability is positively correlated with the body condition of a coastal-pelagic fish. Fish. Oceanogr. 29, 100–110.

Cox, M.K., Heintz, R., 2009. Electrical phase angle as a new method to measure fish condition. Fish. B-NOAA. 107, 477-487.

Cox, M.K., Hartman, K.J., 2005. Nonlethal estimation of proximate composition in fish. Can. J. Fish. Aquat. Sci. 62, 269-275.

Cox, M.K., Heintz, R., Hartman, K., 2011. Measurements of resistance and reactance in fish with the use of bioelectrical impedance analysis: sources of error. Fish. B-NOAA. 109, 34-48.

Dittmar, M., 2003. Reliability and variability of bioimpedance measures in normal adults: effects of age, gender, and body mass. Am. J. Phys. Anthropol. 122, 361-370.

Domínguez-Petit, R., Saborido-Rey, F., Medina, I., 2010. Changes of proximate composition, energy storage and condition of European hake (*Merluccius merluccius*, L. 1758) through the spawning season. Fish. Res. 104, 73-82.

Duncan, M., Craig, S.R., Lunger, A.N., Kuhn, D.D., Salze, G., McLean, E., 2007. Bioimpedance assessment of body composition in cobia *Rachycentron canadum* (L. 1766). Aquaculture 271, 432-438.

Foster, K.R., Lukaski, H.C., 1996. Whole-body impedance - what does it measure? Am. J. Clin. Nutr. 64, 388-396.

Geddes, L.A., Baker, L.E., 1967. The specific resistance of biological material - A compendium of data for the biomedical engineer and physiologist. J. Med. Biol. Eng. 5, 271-293.

Green, A.J., 2001. Mass/length residuals: measures of body condition or generators of spurious results? Ecology 82, 1473-1483.

Gudivaka, R., Schoeller, D., Kushner, R.F., 1996. Effect of skin temperature on multifrequency bioelectrical impedance analysis. J. Appl. Physiol. 81, 838-845.

636 Haard, N.F., 1993. Technological aspects of extending prime quality of seafood: a review. J
637 Aquat. Food Prod. T. 1, 9-27.
638 Hafs, A., Hartman, K., 2015. Development of temperature correction equations for
639 bioelectrical impedance analysis models for brook trout *Salvelinus fontinalis*. J. Fish.
640 Biol. 86, 304-316.
641 Hafs, A.W., Hartman, K.J., 2011. Influence of electrode type and location upon bioelectrical
642 impedance analysis measurements of brook trout. T. Am. Fish. Soc. 140, 1290-1297.
643 Hampton, J., 1986. Effect of tagging on the condition of Southern bluefin tuna, *Thunnus*
644 *maccoyii* (Castlenau). Aust. J. Mar. Fresh. Res. 37, 699-705.
645 Hartman, K.J., Margraf, F.J., 2008. Common relationships among proximate composition
646 components in fishes. J. Fish. Biol. 73, 2352-2360.
647 Hartman, K.J., Margraf, F.J., Hafs, A.W., Cox, M.K., 2015. Bioelectrical impedance analysis:
648 A new tool for assessing fish condition. Fisheries 40, 590-600.
649 Hartman, K.J., Phelan, B.A., Rosendale, J.E., 2011. Temperature effects on bioelectrical
650 impedance analysis (BIA) used to estimate dry weight as a condition proxy in coastal
651 bluefish. Mar. Coast. Fish. 3, 307-316.
652 Hastie, T., Tibshirani, R., Friedman, J., 2001. The Elements of Statistical Learning. Springer
653 series in statistics. Springer, New York.
654 Henry, W.H., Lyle, J.M., 2003. The National Recreational and Indigenous Fishing Survey.
655 Australian Government Department of Agriculture, Fisheries and Forestry. Canberra,
656 Australia.
657 Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric
658 models. Biometrical J. 50, 346–363.
659 Jonsson, N., Jonsson, B., Hansen, L., 1997. Changes in proximate composition and estimates
660 of energetic costs during upstream migration and spawning in Atlantic salmon *Salmo*
661 *salar*. J. Anim. Ecol. 69, 425-436.
662 Kjesbu, O.S., Opdal, A.F., Korsbrekke, K., Devine, J.A., Skjæraasen, J.E., 2014. Making use
663 of Johan Hjort's “unknown” legacy: Reconstruction of a 150-year coastal time-series
664 on northeast Arctic cod (*Gadus morhua*) liver data reveals long-term trends in energy
665 allocation patterns. ICES J. Mar. Sci. 71, 2053-2063.
666 Kyle, U.G., Bosaeus, I., De Lorenzo, A.D., Deurenberg, P., Elia, M., Gómez, J.M., Heitmann,
667 B.L., Kent-Smith, L., Melchior, J.C., Pirlich, M., 2004. Bioelectrical impedance
668 analysis - part I: Review of principles and methods. Clin. Nutr. 23, 1226-1243.
669 Love, R.M., 1970. The chemical biology of fishes. Academic Press, New York.
670 Lowry, M., Molony, B., Keag, M., Penney, A., (2016). Yellowtail kingfish *Seriola lalandi*.
671 Fisheries Research and Development Corporation. Available at
672 http://fish.gov.au/reports/finfish/Pages/yellowtail_kingfish (Verified 1 July 2019).
673 Lukaski, H.C., Johnson, P.E., Bolonchuk, W.W., Lykken, G.I., 1985. Assessment of fat-free
674 mass using bioelectrical impedance measurements of the human body. Am. J. Clin.
675 Nutr. 41, 810-817.
676 Marchello, M., Berg, P., Swantek, P., Tilton, J., 1999. Predicting live and carcass lean using
677 bioelectrical impedance technology in pigs. Livest. Prod. Sci. 58, 151-157.
678 Martinsen, Ø.G., Grimnes, S., Mirtaheri, P., 2000. Non-invasive measurements of post-mortem
679 changes in dielectric properties of haddock muscle–a pilot study. J. Food Eng. 43, 189-
680 192.
681 Miller, P.A., Fitch, A.J., Gardner, M., Hutson, K.S., Mair, G., 2011. Genetic population
682 structure of yellowtail kingfish (*Seriola lalandi*) in temperate Australasian waters
683 inferred from microsatellite markers and mitochondrial DNA. Aquaculture 319, 328-
684 336.

685 Miranda, T., Smith, J., Suthers, I., Mazumder, D., Cruz, D., Schilling, H., Searle, K., Vergés,
686 A., 2019. Convictfish on the move: Variation in growth and trophic niche space along
687 a latitudinal gradient. ICES J. Mar. Sci. 76, 2404–2412.
688 Murphy, B.R., Brown, M.L., Springer, T.A., 1990. Evaluation of the relative weight (*Wr*)
689 index, with new applications to walleye. N. Am. J. Fish. Manag. 10, 85-97.
690 Orr, A., 1920. The retail fish market: Some suggestions for equipping and conducting it. T.
691 Am. Fish. Soc. 49, 107-114.
692 Pilati, A., Vanni, M.J., 2007. Ontogeny, diet shifts, and nutrient stoichiometry in fish. Oikos
693 116, 1663-1674.
694 R Core Team (2017). *R: A language and environment for statistical computing*. Vienna,
695 Austria: R Foundation for Statistical Computing. http:// www.R-project.org/
696 Rätz, H.-J., Lloret, J., 2003. Variation in fish condition between Atlantic cod (*Gadus morhua*)
697 stocks, the effect on their productivity and management implications. Fish. Res. 60,
698 369-380.
699 Stolarski, J., Margraf, F., Carlson, J., Sutton, T., 2014. Lipid and moisture content modeling of
700 amphidromous Dolly Varden using bioelectrical impedance analysis. N. Am. J. Fish.
701 Manag. 34, 471-481.
702 Tierney, M., Hindell, M., Lea, M.A., Tollit, D., 2001. A comparison of techniques used to
703 estimate body condition of southern elephant seals (*Mirounga leonina*). Wildlife Res.
704 28, 581-588.
705 Tracey, S.R., Hartmann, K., Leef, M., McAllister, J., 2016. Capture-induced physiological
706 stress and postrelease mortality for Southern bluefin tuna (*Thunnus maccoyii*) from a
707 recreational fishery. Can. J. Fish. Aquat. Sci. 73, 1547-1556.
708 Van Aerle, R., Runnalls, T., Tyler, C., 2004. Ontogeny of gonadal sex development relative to
709 growth in fathead minnow. J. Fish. Biol. 64, 355-369.
710 Vogt, A., Gormley, T.R., Downey, G., Somers, J., 2002. A comparison of selected rapid
711 methods for fat measurement in fresh herring (*Clupea harengus*). J. Food Compost.
712 Anal. 15, 205-215.
713 Willis, J., Hobday, A.J., 2008. Application of bioelectrical impedance analysis as a method for
714 estimating composition and metabolic condition of southern bluefin tuna (*Thunnus*
715 *maccoyii*) during conventional tagging. Fish. Res. 93, 64-71.
716 Wu, L., Cai, W., Zhang, L., Nakamura, H., Timmermann, A., Joyce, T., McPhaden, M.J.,
717 Alexander, M., Qiu, B., Visbeck, M., 2012. Enhanced warming over the global
718 subtropical western boundary currents. Nat. Clim. Change 2, 161-166.
719